Abstract

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Project Title: Chemical Inhibitors of anti-apoptotic protein Bfl-1

Abstract: DESCRIPTION (provided by applicant): Apoptosis is governed in part by Bcl-2-family proteins. The human genome contains six genes that encode anti-apoptotic members of the Bcl-2 family. Each of these proteins can be inhibited by endogenous proteins that contain a conserved peptidyl domain called BH3. The binding of fluorochrome-conjugated BH3 peptides to anti-apoptotic Bcl-2-family proteins thus provides the basis for construction of Fluorescence Polarization Assays (FPA), suitable for high throughput screening (HTS). Among the anti-apoptotic Bcl-2-family members is Bfl-1 (also known as A1 in mice), a NF-kB-inducible member of the Bcl-2 family. Unlike the other anti-apoptotic members of the Bcl-2 family that have all been successfully ablated in mice, the mouse ortholog of Bfl-1 consists of a cluster of four replicated genes (i.e., four copies of the gene, termed A1a, A1b, A1c, and A1d). Thus, chemical inhibitors are needed as research tools for neutralizing Bfl-1 in human and mouse cells. We propose to identify and optimize chemical inhibitors of Bfl-1. To this end, we have devised procedures for producing multi-milligram quantities of purified recombinant Bfl-1 protein and we have devised a fluorescence polarization assay (FPA), using a Bfl-1-binding synthetic peptide conjugated with FITC. A preliminary screen has been performed of ~10,000 compounds, demonstrating the suitability of this homogeneous assay for the high-throughput environment.

Thesaurus Terms:
High throughput screening, Chemical Inhibitors, anti-apoptotic protein, Bfl-1, BH3, fluorochrome, Bcl-2, Fluorescence Polarization Assay, FPA, NF-kB, A1a, A1b, A1c, A1d, synthetic peptide, FITC

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